Alteration of Oligosaccharide Biosynthesis by Genetic Manipulation of Glycosyltransferases[a]

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INTRODUCTION

Carbohydrate chains can directly mediate or modulate the function of glycoproteins in diverse biological processes.[1] The ability to manipulate the oligosaccharide structures of glycoproteins in order to alter their biological properties would be of obvious value. This is particularly relevant with regard to biologically important molecules such as growth factors, hormones, and other therapeutic agents that are being produced in cultured cells. Altering the sugar chains of these glycoproteins may improve their therapeutic value by increasing their efficacy, altering their circulatory half-lives, and/or increasing their target specificity. In addition, altered glycosylation of cell-surface components may provide insight to the precise roles that cell-surface glycoconjugates play in processes such as migration, adhesion, development, and malignancy.

Several methods have evolved to alter glycosylation in cells. These have included the use of reagents that inhibit glycosylation as well as inhibitors of glycosylation processing.[2] These inhibitors have been widely used to study the sugar chains of glycoconjugates, but many of these reagents are toxic to cells, and in some instances, their effects are only partial. Another approach has been to use mutagenized cells that are resistant to the toxic effects of specific lectins due to deficiencies in corresponding glycosylation reactions.[3] One limitation of this approach is that mutants are not generated at each step of the biosynthetic pathway. In addition, both of these approaches are of limited use for obtaining large, complex oligosaccharides, inasmuch as they result in the formation of incomplete or truncated carbohydrate structures.

One method that overcomes these limitations and that allows one to selectively manipulate oligosaccharide structure is to express cloned genes for glycosyltransferases into mammalian cells. As more genes for these enzymes are cloned, the possibilities for altering the biosynthetic pathways of oligosaccharide in cells could be substantial. It is this new approach that will be the focus of this review.

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alized to the endoplasmic reticulum and se enzymes catalyze the transfer of sugars termediates to mono- and oligosaccharide different glycosyltransferases have been e of these enzymes share common sub- mology. The topology and domain struc- s, however, are remarkably similar in that ontaining short cytoplasmic domains and ailability of cDNAs for many of these them into host cells and to subsequently

netic manipulation of glycosyltransferases xpression of glycosyltransferases that are ogenous glycosyltransferases), and over- cells containing endogenous activities of ferases). The first approach has been the ng for alterations in glycosylation is rela- ovel oligosaccharides on the cell surface second approach, on the other hand, re- because increasing the levels of glycosyl- rate different types, but rather, different

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osyltransferases

n of exogenous glycosyltransferases has ation, such as fucosylation and sialylation. ies that have used this approach to modify

catalyzes the transfer of fucose (Fuc) from

fucose to galactose (Gal) residues, antigen. Similar to the studies with α1,2 FT into COS-1 cells, which l of H blood group antigen.[10]

C. α1,3 Galactosyltransferase UDP-Gal to terminal Gal residues GT into CHO cells results in the rides.[11] Furthermore, the expressio in terminal sialylation of sugar chai competition between different glyc

D. α2,6 Sialyltransferase (α2, from CMP-SA to terminal Gal re protein into CHO cells results in chains.[12] As was the case for exan between the transfected sialyltransf SA in an α2,3 linkage to Gal.

E. β1,3 Galactosyltransferase (GlcNAc residues in the biosynthes mine chains. This class of oligosac importance because it serves as the Transfection of the cDNA encodin the biosynthesis of type 1 *N*-ace addition, many of these chains we contrast to the studies described expression of glycosyltransferase during elongation) can also alter

Endogen

To date, the only example of glycosyltransferase is that of β1,4 lyzes the transfer of Gal from UI synthesis of type 2 (Gal β1,4 Gl complex oligosaccharides. β1,4 G in that one gene encodes two simil

fucose to galactose (Gal) residues, resulting in the formation of the H blood group antigen. Similar to the studies with α1,3 FT, the introduction of the cloned gene for α1,2 FT into COS-1 cells, which lack this enzymic activity, results in the formation of H blood group antigen.[10]

C. α1,3 Galactosyltransferase (α1,3 GT) catalyzes the transfer of Gal from UDP-Gal to terminal Gal residues of oligosaccharide chains. Transfection of α1,3 GT into CHO cells results in the expression of α1,3 Gal-containing oligosaccharides.[11] Furthermore, the expression of this enzyme results in a concomitant decrease in terminal sialylation of sugar chains in these cells. Thus, this study also shows that competition between different glycosyltransferases can affect glycosylation *in vivo.*

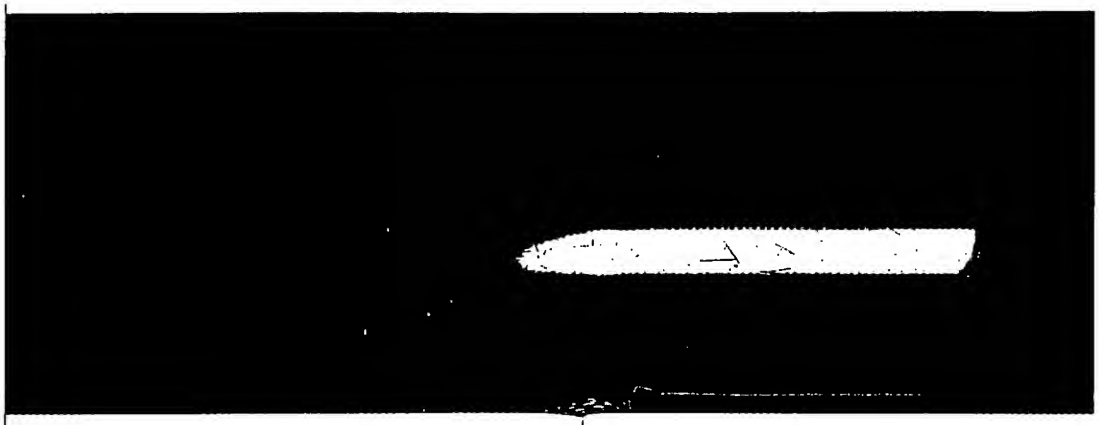
D. α2,6 Sialyltransferase (α2,6 ST) catalyzes the transfer of sialic acid (SA) from CMP-SA to terminal Gal residues. Transfection of the cDNA encoding this protein into CHO cells results in the expression of appropriately sialylated sugar chains.[12] As was the case for example C above, there is competition for substrates between the transfected sialyltransferase and a similar endogenous enzyme that adds SA in an α2,3 linkage to Gal.

E. β1,3 Galactosyltransferase (β1,3 GT) catalyzes the transfer of Gal to terminal GlcNAc residues in the biosynthesis of type 1 (Gal β1,3 GlcNAc) *N*-acetyllactosamine chains. This class of oligosaccharides is of particular biological and structural importance because it serves as the core structure for various blood group activities. Transfection of the cDNA encoding this enzyme into human colonic cells results in the biosynthesis of type 1 *N*-acetyllactosamine-containing oligosaccharides.[13] In addition, many of these chains were further modified with SA and Fuc residues. In contrast to the studies described above, this study is unique in showing that the expression of glycosyltransferase acting early in oligosaccharide biosynthesis (*i.e.*, during elongation) can also alter glycosylation.

Endogenous Glycosyltransferase

To date, the only example of over-expression of an endogenously expressed glycosyltransferase is that of β1,4 galactosyltransferase (β1,4 GT). β1,4 GT catalyzes the transfer of Gal from UDP-Gal to terminal GlcNAc residues in the biosynthesis of type 2 (Gal β1,4 GlcNAc) *N*-acetyllactosamine cores of all *N*-linked complex oligosaccharides. β1,4 GT is unique among the cloned glycosyltransferases in that one gene encodes two similar forms of the enzyme that differ by an additional 13 amino acids at the cytoplasmic N-terminus of the long form that is not present in the short form of the enzyme.[14,15] Both the long and the short form are localized primarily in the Golgi complex. However, the long form of GT is also targeted to the plasma membrane,[15] where it associates with the cytoskeleton and functions as a cell-adhesion molecule.[16]

Because of the central role this enzyme plays in the biosynthesis of *N*-acetyllactosamine–containing oligosaccharides, the effects of overexpressing this enzyme were explored in detail. Transfection of the cDNAs encoding the two forms of β1,4 GT into F9 embryonal carcinoma cells expressing endogenous enzyme results in a threefold increase in total β1,4 GT activity compared to control cells.[17] Analysis of [^{3}H]Gal-labeled glycoproteins and glycopeptides by a variety of methods revealed



ifferences in glycosylation. Similar anal-
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inasmuch as an excess of substrate was
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this enzyme is responsible for a terminal
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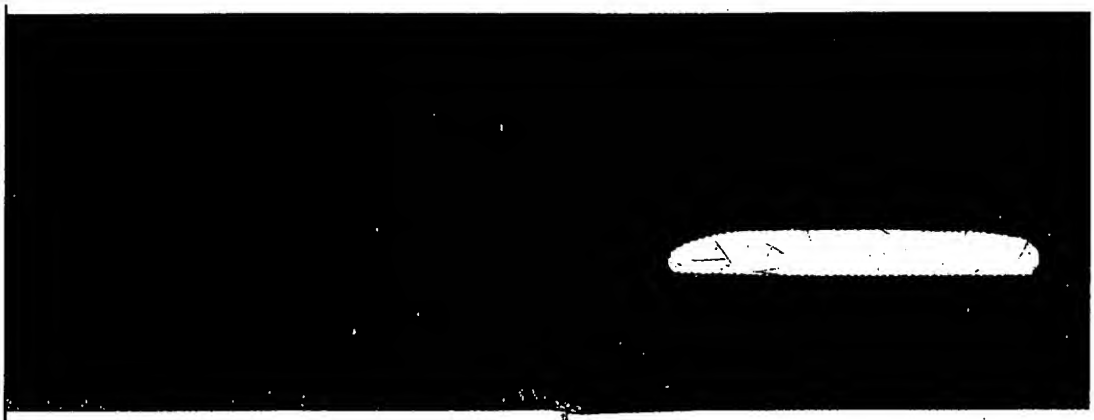

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